Auxin Production by Plant-Pathogenic Pseudomonads and Xanthomonads

WILLIAM F. FETT,* STANLEY F. OSMAN, AND MICHAEL F. DUNN

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, Pennsylvania 19118

Received 30 January 1987/Accepted 26 May 1987

Pathogenic strains of *Xanthomonas campestris* pv. *glycines* which cause hypertrophy of leaf cells of susceptible soybean cultivars and nonpathogenic strains which do not cause hypertrophy were compared for their ability to produce indole compounds, including the plant hormone indole-3-acetic acid (IAA) in liquid media with or without supplementation with L-tryptophan. Several additional strains of plant-pathogenic xanthomonads and pseudomonads were also tested for IAA production to determine whether in vitro production of IAA is related to the ability to induce hypertrophic growth of host tissues. Indoles present in culture filtrates were identified by thin-layer chromatography, high-performance liquid chromatography, UV spectroscopy, mass spectroscopy, and gas chromatography-mass spectrometry and were quantitated by high-performance liquid chromatography. All strains examined produced IAA when liquid media were supplemented with L-tryptophan. The highest levels of IAA were found in culture filtrates from the common bean pathogen *Pseudomonas syringae* pv. *syringae*, and this was the only bacterium tested which produced IAA without addition of tryptophan to the medium. Additional indoles identified in culture filtrates of the various strains included indole-3-lactic acid, indole-3-aldehyde, indole-3-acetamide, and *N*-acetyltryptophan. Pseudomonads and xanthomonads could be distinguished by the presence of *N*-acetyltryptophan, which was found only in xanthomonad culture filtrates.

The ability to produce the plant hormone indole-3-acetic acid (IAA) is widespread among fungi and bacteria (11, 33). Microorganisms which commonly inhabit the aerial or subterranean surfaces of plants have been shown to be capable of IAA synthesis (41). Such microorganisms include rhizobia (1), mycorrhizal fungi (5), pseudomonads (23, 25), *Azospirillum brasilense* (39), and *Azotobacter paspali* (2). Fungi and bacteria which cause plant disease have also been reported to produce IAA (11, 31), but in most instances the identity of IAA was not rigorously confirmed by modern analytical methods and the importance of in planta IAA production by most of these organisms for the disease process is not clear. Three plant pathogens for which microbial IAA production has been shown to be important for pathogenicity are *Pseudomonas syringae* pv. *savastanoi*, *Agrobacterium tumefaciens*, and *Agrobacterium rhizogenes*. *P. syringae* pv. *savastanoi* induces galls or knots on stems and leaves of infected hosts, and the ability to induce hypertrophic growth is dependent on IAA production by the pathogen (4, 35, 37). IAA production encoded by bacterial genes has also been shown to be required for tumor formation by *A. tumefaciens* (22) and for induction of tumors and roots by *A. rhizogenes* (24).

The soybean pathogen *Xanthomonas campestris* pv. *glycines* causes bacterial pustule disease of susceptible cultivars, characterized by the formation of small circular lesions with erumpent centers (34). Pustule formation is primarily due to hypertrophy of host mesophyll cells (10, 14, 43), indicating a possible involvement of elevated auxin levels. Millar (R. Millar, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1955) reported that *X. campestris* pv. *glycines* is capable of auxin production from tryptophan, based on colorimetric assays and on bioassays using *Avenae* coleoptiles. Auxin production by *X. campestris* pv. *glycines* in planta may be responsible for the distinctive symptomology of bacterial pustule disease.

The purpose of this study was to confirm that *X. campestris* pv. *glycines* is capable of auxin synthesis, to identify and quantitate the tryptophan metabolites, and to determine whether nonpathogenic strains have lost the ability to produce auxin. In addition, we wished to determine whether plant-pathogenic pseudomonads and xanthomonads which are not reported to cause hypertrophy of host tissues are capable of auxin synthesis.

(A preliminary report of part of this work has been presented [W. F. Fett, S. F. Osman, and M. F. Dunn, Phytopathology **76**:1011, 1986].)

MATERIALS AND METHODS

Materials. All indole standards except for indole-3-lactic acid (ILA) were obtained from Sigma Chemical Co., St. Louis, Mo. ILA was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.

Bacteria. The sources and origins of the bacterial strains are given in Table 1. *X. campestris* pv. *glycines* strains B83, 1717, XP175, R12, B99, and S-9-4 are pathogenic, strain 1714 is of reduced virulence, and strains S-9-8, 1135, 1136, and 1716 are nonpathogenic variants (7).

Growth media and culture conditions. Bacteria were maintained on potato dextrose agar (Difco Laboratories, Detroit, Mich.) (xanthomonads), *Pseudomonas* agar F (Difco) (fluorescent pseudomonads), or nutrient dextrose agar (Difco nutrient agar plus 10 g of Difco dextrose per liter and 5 g of Difco yeast extract per liter) (nonfluorescent pseudomonads) at 4°C with monthly transfer. For long-term storage, strains were freeze-dried in double-strength skim milk.

Both a complex and a completely defined liquid medium were used for the xanthomonads. The complex medium was

* Corresponding author.

TABLE 1. Sources and origins of bacterial strains

Bacterium	Strain	Source[a]	Origin (host)
Pseudomonas avenae	VS-1	R. D. Gitaitis	*Paspalum urvillei*
Pseudomonas caryophylli	CUCPB 1268	R. S. Dickey	*Dianthus caryophyllus*
Pseudomonas marginalis	HT041B	C. H. Liao	*Spinacia oleracea*
Pseudomonas syringae			
pv. *glycinea*	NCPPB 2159	NCPPB	*Glycine max*
pv. *phaseolicola*	At	D. M. Webster	*Phaseolus vulgaris*
pv. *syringae*	Meyer	D. M. Webster	*Phaseolus vulgaris*
pv. *tabaci*	Pt113	R. D. Durbin	*Nicotiana tobacum*
pv. *tomato*	84-86	R. D. Gitaitis	*Lycopersicon esculentum*
Pseudomonas viridiflava	SF0312B	C. H. Liao	*Cucurbita pepo*
Xanthomonas campestris			
pv. *campestris*	MPA	R. D. Gitaitis	*Brassica oleracea* var. *capitata*
pv. *glycines*	B83	L. Ferreira	*Glycine max*
	NCPPB 1717	NCPPB	*Glycine max*
	XP175	M. P. Starr	*Glycine max*
	R12	J. Dunleavy	*Glycine max*
	B99	L. Ferreira	*Glycine max*
	NCPPB 1714	NCPPB	*Neonotönia wightii*
	S-9-4	W. F. Fett	*Glycine max*
	S-9-8	W. F. Fett	*Glycine max*
	NCPPB 1135	NCPPB	*Glycine max*
	NCPPB 1136	NCPPB	*Neonotönia wightii*
	NCPPB 1716	NCPPB	*Neonotönia wightii*
pv. *malvacearum*	D	G. R. Lazo	*Gossypium hirsutum*
pv. *mannihotis*	3.25L	UWCC	*Manihot esculenta*
pv. *pelargonii*	L126	UWCC	Not known
pv. *phaseoli*	BSB	R. D. Gitaitis	*Phaseolus vulgaris*
	Xcf27	A. W. Saettler	*Phaseolus vulgaris*
pv. *pruni*	84-22	R. D. Gitaitis	*Prunus persica*
pv. *raphani*	70-5	R. E. Stall	
pv. *vesicatoria*	84-79	R. D. Gitaitis	*Lycopersicon esculentum*
pv. *vignicola*	432	R. D. Gitaitis	*Vigna unguiculata*
Undesignated	CJ092	C. H. Liao	*Cucumis sativa*
	CJ093	C. H. Liao	*Cucumis sativa*
	PP061	C. H. Liao	*Carica papaya*
	PF083	C. H. Liao	*Capsicum annuum*
	TJ071	C. H. Liao	*Lycopersicon esculentum*

[a] NCPPB, National Collection of Plant Pathogenic Bacteria, Hatching Green, England; UWCC, University of Wisconsin Department of Plant Pathology Culture Collection, Madison.

yeast-salts-glycerol (YSG) and contained the following (per liter) : $NH_4H_2PO_4$, 0.5 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 5.0 g; yeast extract (Difco), 5.0 g; and glycerol, 10 g. The pH was adjusted to 7.0 before sterilization. The composition of the defined medium (S-D) was based on the study of Souw and Demain (36). The medium contained (per liter) KH_2PO_4, 6.8 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $(NH_4)_2SO_4$, 2.0 g; citrate, 2.0 g; H_3BO_3, 0.006 g; ZnO, 0.006 g; $FeCl_3 \cdot 6H_2O$, 0.0024 g; $CaCO_3$, 0.02 g; and HCl, 0.13 ml. The pH was adjusted to 7.0 before sterilization. Glucose (10 g) was sterilized separately and added to the cooled medium. DL-Methionine and L-tryptophan were filter sterilized and added to the cooled medium to give final concentrations of 0.02% (wt/vol) and 0.05% (wt/vol), respectively.

The semisynthetic liquid medium described by Brugger and Keen (3), with potassium phosphate substituted for yeast extract, was used for the pseudomonads. The pH was adjusted to 7.0 before sterilization. Glucose was sterilized separately, L-tryptophan was filter sterilized, and both were added to the cooled medium to give final concentrations of 1% (wt/vol) and 0.05% (wt/vol), respectively.

Xanthomonads were grown on nutrient or S-D agar, and pseudomonads were grown on *Pseudomonas* agar F or nutrient dextrose agar, all without added L-tryptophan, overnight at 28°C. A loopful of cells was used to inoculate liquid medium for starter cultures. After being incubated overnight at 28 to 30°C with shaking, either the cultures were diluted to give an optical density at 600 nm (OD_{600}) of approximately 1.0 or, for less dense cultures, cells were first concentrated by centrifugation and then suspended in sterile water and the turbidity was adjusted to give an OD_{600} of approximately 1.0. For initial experiments, 5 ml of the cell suspension was used to inoculate 500 ml of liquid medium in a 2,800-ml Fernbach flask. For later experiments, 50 or 75 ml of liquid medium in 300-ml sidearm flasks was inoculated with 0.5 or 0.75 ml, respectively, of cell suspension. The flasks were shaken (200 rpm) in dim light or in the dark at 28 to 30°C, and growth was determined by monitoring the OD_{600}. When the cultures reached the stationary phase, cells were removed by centrifugation followed by filtration (pore size, 0.45 μm). In most experiments, the cells were washed three times with saline adjusted to pH 7.0 and then freeze-dried and weighed.

Extraction, identification, and quantitation of indoles. For some experiments, neutral, acidic, and basic indoles were sequentially extracted from culture filtrates by partitioning the filtrates with ethyl acetate at pHs of 8.0, 3.0, and 11.0, respectively (44). The pH was adjusted upward with $NaHCO_3$ and downward with 1 N HCl. In later experiments, extractions were done at pH 3.0 only. Anhydrous sodium sulfate was added to dry the ethyl acetate fractions. After the fractions were allowed to sit overnight at room temperature,

TABLE 2. Indole production by *X. campestris* pv. *glycines* in liquid media supplemented with 0.05% L-tryptophan

Medium	Strain	Amt (mean ± SD)[a] produced									
		IAA		IAld		ILA		IAM		*N*-AcTrp	
		μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/mg
YSG	B83	1.91	ND[b]	0.57	ND	12.23	ND	<d[c]		3.68	ND
	XP175	0.28 ± 0.12	0.18 ± 0.06	ND	ND	4.82 ± 2.55	2.99 ± 1.36	<d		ND	ND
	R12	0.22 ± 0.03	0.17 ± 0.03	ND	ND	2.25 ± 0.40	1.67 ± 0.33	<d		ND	ND
	B99	0.24 ± 0.05	0.19 ± 0.04	ND	ND	4.91 ± 0.98	3.79 ± 0.73	<d		ND	ND
	1714	0.36 ± 0.15	0.25 ± 0.10	ND	ND	5.36 ± 1.21	3.84 ± 0.83	<d		ND	ND
	S-9-4	0.26 ± 0.07	0.18 ± 0.05	ND	ND	2.58 ± 0.31	1.84 ± 0.23	<d		ND	ND
	S-9-8	6.37	ND	2.99	ND	0.72	ND	<d		3.46	ND
	1135	0.94	ND	0.52	ND	6.78	ND	<d		1.28	ND
	1136	1.48	ND	0.86	ND	2.29	ND	<d		0.87	ND
	1716	1.02	ND	0.52	ND	4.24	ND	<d		1.15	ND
Completely defined	B83	1.85	ND	0.70	ND	5.90	ND	1.06	ND	3.33	ND
	1717	1.66	ND	1.14	ND	38.65	ND	0.06	ND	10.24	ND
	S-9-8	1.30	ND	0.50	ND	0.26	ND	0.06	ND	1.31	ND

[a] Values without standard deviations are from single experiments. Values with standard deviations are averages of data from two experiments. All values are uncorrected for recovery efficiencies.
[b] ND, Not determined.
[c] <d, Less than the detection limit of 0.01 μg/ml.

Na_2SO_4 was removed by filtration and rinsed with a small volume of ethyl acetate. The combined ethyl acetate fractions were then taken to dryness under a stream of nitrogen.

The level of IAA in the culture filtrates before extraction with ethyl acetate was estimated by the colorimetric assay of Gordon and Weber (9). The ethyl acetate fractions were examined for IAA and additional indoles by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Dried fractions were taken up in methanol, and any particulate matter was removed by filtration (pore size, 0.20 μm).

For TLC, samples were applied to Silica Gel G 250-μm TLC plates (Analtech, Inc., Newark, Del.), and the plates were irrigated with chloroform-methanol-acetic acid (80:15:5, vol/vol) or benzene-ethyl acetate-acetic acid (70:25:5, vol/vol). After the plates were air dried, indoles were located by spraying with Ehrlich's reagent (Sigma) (18). Indole-3-aldehyde (IAld) gave variable color formation with Ehrlich's reagent and was located by spraying with 0.4% 2,4-dinitrophenylhydrazine in 2 N HCl (2,4-DNP) (18).

For HPLC, a Hewlett-Packard (Hewlett-Packard Co., Avondale, Pa.) HP 1090 system equipped with a Model 1040A Diode-Array UV/visible detector system was used. Indoles were separated on a Supelcosil LC-18 reverse-phase column (150 by 4.6 mm [inside diameter]; particle size, 3 μm; Supelco, Inc., Bellefonte, Pa.). The column temperature was maintained at 50°C. Initially, neutral and acidic indoles were eluted isocratically with 20% methanol in water (pH 3.0), but retention times were not stable. More stable retention times were obtained by substituting 1% acetic acid for water at pH 3.0. However, under these conditions *N*-acetyltryptophan (*N*-AcTrp) and IAld coeluted. Basic indoles were eluted isocratically with 20% methanol in 0.1 M ammonium carbonate. The flow rate was 1 ml/min. The A_{280} was monitored, and UV absorption profiles of peak materials were obtained. Indoles were quantified by reference to a standard curve of peak area versus concentration generated for external standards. For semipreparative HPLC, a Dynamax C_{18} reverse-phase column (250 by 10 mm [inside diameter]; particle size, 8 μm; Rainin Instrument Co., Inc., Woburn, Mass.) was substituted for the analytical column. Experimental conditions were the same as those described above except that the methanol concentration was increased to 30% and the flow rate was increased to 3 ml/min.

Confirmation of the identity of the indoles, except for indole-3-acetamide (IAM), was obtained by gas chromatography-mass spectrometry. Indoles were converted to trimethylsilyl (TMS) derivatives with bis(trimethylsilyl) trifluoroacetamide (Supelco) by heating them at 70°C for 30 min. The TMS derivatives were analyzed on a Hewlett-Packard 5990B gas chromatograph-mass spectrometer by using an OV-101 25-m capillary column in a temperature programming mode (150 to 250°C at 4°C per min). Identification was confirmed by comparison of the results with those for TMS derivatives of standards. Confirmation of the identity of IAM was obtained by direct probe insertion mass spectrometry using an underivatized sample.

RESULTS

Indole compound production by xanthomonads. Good growth was exhibited by all xanthomonads in YSG liquid medium, with OD_{600} readings ranging from approximately 2 to 4 at 24 h. Growth in S-D defined liquid medium was much slower, with OD_{600} readings between 0.7 and 1.5 at 40 h. All pathogenic and nonpathogenic strains of *X. campestris* pv. *glycines* tested produced the plant hormone IAA, as well as additional indole compounds, when grown in complex or defined liquid medium supplemented with L-tryptophan (Table 2). No indole compounds were detected in culture filtrates when tryptophan was omitted from the media.

TLC of ethyl acetate extracts indicated the presence of IAA, ILA, *N*-AcTrp, and IAld. The indoles, except for IAld, were located as blue spots on TLC plates after spraying with Ehrlich's reagent. IAld did not give good color formation with Ehrlich's reagent but did give an easily discernible brown spot when plates were sprayed with 2,4-DNP. All the indole compounds gave UV spectra (15) and retention times identical to those of corresponding standards after separation by HPLC. When HPLC conditions were such that IAld and *N*-AcTrp coeluted, no quantitative data could be obtained for these two compounds. However, the presence of

TABLE 3. Indole production by additional *X. campestris* pathovars in liquid media supplemented with 0.05% L-tryptophan

Medium	Pathovar	Strain	Amt (mean ± SD)[a] produced								
			IAA		IAld		ILA		IAM	*N*-AcTrp	
			μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/ml	μg/mg
YSG	*campestris*	MPA	0.31 ± 0.11	0.24 ± 0.05	ND	ND	0.16 ± 0.02	0.13 ± 0.04	<d	ND	ND
	malvacearum	D	0.16 ± 0.08	0.09 ± 0.04	ND	ND	0.66 ± 0.35	0.39 ± 0.18	<d	ND	ND
	mannihotis	3.25L	0.13 ± 0.00	0.15 ± 0.04	ND	ND	1.60 ± 0.32	1.84 ± 0.07	<d	ND	ND
	pelargonii	L126	0.23 ± 0.03	0.17 ± 0.00	ND	ND	0.20 ± 0.04	0.15 ± 0.01	<d	ND	ND
	phaseoli	BSB	0.56 ± 0.35	0.35 ± 0.18	ND	ND	0.19 ± 0.05	0.12 ± 0.02	<d	ND	ND
		Xcf27	0.17 ± 0.01	0.19 ± 0.01	ND	ND	0.73 ± 0.02	0.81 ± 0.06	<d	ND	ND
	pruni	84-22	0.60 ± 0.03	0.30 ± 0.01	ND	ND	0.12 ± 0.18	0.07 ± 0.10	<d	ND	ND
	raphani	70-5	0.67 ± 0.01	0.49 ± 0.05	ND	ND	0.10 ± 0.15	0.07 ± 0.10	<d	ND	ND
	vesicatoria	84-79	0.16 ± 0.02	0.19 ± 0.01	ND	ND	0.79 ± 0.21	0.95 ± 0.08	<d	ND	ND
	vignicola	432	0.21 ± 0.04	0.13 ± 0.02	ND	ND	1.28 ± 0.24	0.82 ± 0.11	<d	ND	ND
	Undesignated	CJ092	0.50 ± 0.24	0.29 ± 0.12	ND	ND	0.30 ± 0.04	0.17 ± 0.01	<d	ND	ND
		CJ093	0.39 ± 0.20	0.24 ± 0.10	ND	ND	0.16 ± 0.07	0.10 ± 0.06	<d	ND	ND
		PP061	0.42 ± 0.19	0.26 ± 0.10	ND	ND	0.23 ± 0.02	0.15 ± 0.02	<d	ND	ND
		PF083	0.39 ± 0.20	0.24 ± 0.10	ND	ND	0.16 ± 0.08	0.10 ± 0.06	<d	ND	ND
		TJ071	0.40 ± 0.21	0.27 ± 0.12	ND	ND	0.22 ± 0.00	0.15 ± 0.01	<d	ND	ND
Completely defined	*pelargonii*	L126	0.16	0.43	ND	ND	<d		<d	0.16	0.43
	phaseoli	BSB	1.25	0.96	0.55	0.42	<d		<d	1.55	1.20
		Xcf27	0.11	0.18	0.09	0.15	0.37	0.60	<d	1.02	1.69

[a] See Table 2, footnotes *a*, *b*, and *c*.

IAld in all samples was indicated by its characteristic UV profile, which differs from those of the other indoles encountered in the culture filtrates due to the presence of a double bond in conjugation with the ring structure (15). Peak material corresponding to IAM was collected off the HPLC column and examined by TLC. The collected peak material gave a single spot which cochromatographed with authentic IAM with the two solvent systems and gave identical color formation after spraying with Ehrlich's reagent. Positive identification of the TMS derivatives of IAA, IAld, ILA, and *N*-AcTrp was obtained by comparison of mass spectra with those of standards. The spectrum of the IAA derivative contained the following characteristic ions: *m/z* 319 (M^+), 202 (B^+). Characteristic ions for the other indole derivatives were as follows: IAld, *m/z* 217 (M^+,B^+); ILA, *m/z* 421 (M^+), 202 (B^+), and 73 (B^+); *N*-AcTrp, *m/z* 390 (M^+), 202 (B^+). These findings are consistent with other reports (1). Culture filtrates of strains B83 and S-9-8 grown in complex medium and of strains 1717, B83, and S-9-8 grown in defined medium were examined for the presence of basic indoles. No basic indoles were found.

HPLC analysis of YSG medium alone showed the presence of 0.064 μg of IAA per ml and 0.057 μg of IAld per ml but no ILA or *N*-AcTrp. HPLC analysis of S-D medium alone indicated the absence of any contaminating indoles. The recovery efficiency based on extractions of liquid medium immediately after the addition of IAA at 20 μg/ml averaged 60% for six separate determinations. Also, no decomposition of IAA to ILA, IAld, IAM, or *N*-AcTrp was found when flasks containing S-D medium with added IAA were shaken for 48 h in dim light before extraction. The recovery efficiencies for IAA after shaking for 48 h were similar to those found when media were extracted immediately after the addition of IAA.

In one experiment the effect of substituting L-tyrosine (Sigma) for L-tryptophan in S-D medium on indole production by strains B83 and S-9-8 was determined. No indoles were produced by either strain under these conditions based on TLC and HPLC analyses.

Since pathogenic strains of *X. campestris* pv. *glycines* cause hypertrophic growth, which may indicate a hyperauxinic condition of host tissue, we examined other *X. campestris* pathovars which have not been reported to induce hypertrophy of host tissues to determine whether such pathogens could also produce IAA in vitro. TLC, HPLC, and gas chromatography-mass spectrometry analyses indicated that strains representing nine additional pathovars of *X. campestris* and five strains of soft-rotting *X. campestris* which have not been assigned to a particular pathovar (20) produced IAA, IAld, ILA, and *N*-AcTrp when grown in liquid medium supplemented with L-tryptophan (Table 3). No IAM was found in culture filtrates of these strains. No indole compounds were detected for any of the strains when tryptophan was omitted from the media.

Indole compound production by pseudomonads. Nine strains of phytopathogenic pseudomonads including *P. marginalis* and *P. viridiflava*, two soft-rotting bacteria (20a), were tested for indole compound production in a semisynthetic liquid medium. When the medium was supplemented with L-tryptophan, TLC and HPLC analyses indicated the presence of IAA, IAM, ILA, and IAld in culture filtrates (Table 4). Gas chromatography-mass spectrometry analysis confirmed the identity of IAA, ILA, and IAld. To confirm the presence of IAM, 3 liters of culture filtrate of *P. syringae* pv. *syringae* Meyer were extracted at pH 8 with ethyl acetate. IAM was purified by semipreparative HPLC. The material collected off the column which corresponded to the retention time and UV spectrum of authentic IAM also cochromatographed with authentic IAM on TLC plates with the two solvent systems and gave identical color formation after being sprayed with Ehrlich's reagent. Positive identification was by mass spectrometry using direct probe insertion. Characteristic ions were *m/z* 174 (M^+) and 130 (B^+) (13). Other abundant ions were *m/z* 103 and 77.

P. syringae pv. *syringae* Meyer produced exceptionally high amounts of IAA. When L-tryptophan was omitted from the medium, only *P. syringae* pv. *syringae* Meyer still produced IAA (4.34 μg/ml or 2.52 μg/mg [dry weight],

TABLE 4. Indole production by phytopathogenic pseudomonads in liquid medium supplemented with 0.05% L-tryptophan

Pseudomonad	Strain	Amt (mean ± SD)[a] produced							
		IAA		IAld		ILA		IAM	
		μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/mg	μg/ml	μg/mg
P. avenae	VS-1	0.90 ± 0.24	1.43 ± 0.57	0.60 ± 0.06	0.93 ± 0.05	0.48 ± 0.04	0.75 ± 0.16	<d	
P. caryophylli	CUCPB 1268	0.69 ± 0.17	0.81 ± 0.16	0.56 ± 0.08	0.66 ± 0.06	0.07 ± 0.10	0.08 ± 0.11	<d	
P. marginalis	HT041B	0.52 ± 0.40	0.42 ± 0.41	<d		<d			
P. syringae									
pv. *glycinea*	NCPPB 2159	1.06 ± 0.12	0.67 ± 0.06	1.26 ± 0.93	0.80 ± 0.58	1.45 ± 1.14	0.93 ± 0.75	<d	
pv. *phaseolicola*	At	1.68 ± 1.32	1.20 ± 1.07	1.63 ± 0.45	1.12 ± 0.47	1.35 ± 0.70	0.98 ± 0.66	0.43 ± 0.28	0.31 ± 0.23
pv. *syringae*	Meyer	40.72 ± 11.17	29.64 ± 2.33	1.13 ± 0.46	0.88 ± 0.51	2.38 ± 0.21	1.76 ± 0.20	2.82 ± 0.53	2.15 ± 0.81
pv. *tabaci*	Pt113	2.50 ± 1.26	2.09 ± 1.05	2.52 ± 0.14	2.10 ± 0.13	2.98 ± 1.45	2.49 ± 1.21	<d	
pv. *tomato*	84-86	1.27 ± 0.36	1.12 ± 0.13	0.84 ± 0.41	0.86 ± 0.69	4.48 ± 1.29	4.42 ± 2.87	<d	
P. viridiflava	SF0312B	2.13 ± 1.18	1.93 ± 1.30	2.19 ± 0.52	1.95 ± 0.75	1.76 ± 0.40	1.56 ± 0.57	<d	

[a] See Table 2, footnotes *a* and *c*.

uncorrected for recovery efficiency). This strain did not produce detectable levels of the other indole compounds under these conditions.

DISCUSSION

This study confirmed our suspicion, based on light and electron microscopy observations (10, 14, 43) and the report by Millar (Ph.D. thesis), that *X. campestris* pv. *glycines* is capable of synthesizing the plant hormone IAA, as well as several additional indoles, from L-tryptophan. ILA has been shown to have auxinlike activity on wheat coleoptiles (27), but to our knowledge there are no reports of auxinlike activity for IAM or *N*-AcTrp.

The relationship of IAA synthesis to the pathogenicity of *X. campestris* pv. *glycines* is not clear. All nonpathogenic strains proved capable of IAA synthesis in vitro, and the values for the nonpathogenic strain S-9-8 were equal to or greater than the values for the pathogenic strains. Similarly, a nonpathogenic mutant of *Pseudomonas solanacearum* was reported to produce fivefold greater amounts of IAA in vitro than did its parent pathogenic strain (32). Liu and Kado (21) reported a much greater differential in levels of IAA synthesis between virulent (higher) and avirulent (lower) strains of *A. tumefaciens* when tyrosine was substituted for tryptophan in a completely defined liquid growth medium, but substituting tyrosine for tryptophan in our study did not allow for IAA synthesis by *X. campestris* pv. *glycines*. The inability of *X. campestris* pv. *glycines* to produce IAA in vitro without the addition of high levels of tryptophan to the media casts serious doubts on the importance of bacterial IAA synthesis in planta for symptom production.

All pathogenic strains of *X. campestris* pv. *glycines*, isolated from diverse geographical locations, have been found to contain an identical or nearly identical plasmid, based on restriction endonuclease digestion patterns (11a). It is possible that the genes coding for IAA synthesis are borne on this highly conserved plasmid. However, all nonpathogenic strains of *X. campestris* pv. *glycines* are devoid of plasmids (11a). The ability of these strains to synthesize IAA indicates that the IAA genes are chromosomal.

Representatives of nine additional pathovars of *X. campestris* and five soft-rotting *X. campestris* strains (20) produced the same indole compounds as did *X. campestris* pv. *glycines*. To our knowledge none of these strains is known to induce hypertrophy of host tissues. Production of IAA by xanthomonads other than *X. campestris* pv. *glycines* has been previously reported for *X. campestris* pv. *campestris* (28), *X. beticola* (sic) (8), *X. campestris* pv. *citri* (12), and *X. campestris* pv. *phaseoli* (Millar, Ph.D. thesis). However, the identification of IAA was confirmed by mass spectrometry only for *X. campestris* pv. *campestris* (28). Of these bacteria only *X. campestris* pv. *citri* has been reported to induce hypertrophy of host tissues (16).

Strains representing nine different plant pathogenic pseudomonads were also found capable of induced synthesis of IAA and additional indole compounds in liquid culture media supplemented with L-tryptophan. Only the *P. syringae* pv. *syringae* strain was capable of constitutive IAA synthesis, but IAA levels were increased 10-fold by the addition of L-tryptophan to the medium. The pseudomonads tested differed from the xanthomonads in that no *N*-AcTrp was found in culture filtrates of the former. The pseudomonads used in this study have not been reported to cause hypertrophy of host tissues. Production of IAA by plant-pathogenic pseudomonads in addition to *P. syringae* pv. *savastanoi* has been previously reported for *P. syringae* pv. *syringae* (6), *P. solanacearum* (32), *P. syringae* pv. *eriobotryae* (12), and *P. syringae* pv. *morsprunorum* (6). Of these pseudomonads only *P. syringae* pv. *eriobotryae* induces hypertrophic growth (42). In these reports, identification of IAA was based solely on paper chromatography, reaction with chromogenic reagents, and bioassay, except for *P. solanacearum*. IAA production by *P. syringae* pv. *savastanoi* and *P. solanacearum* is constitutive, but increased levels of IAA are produced when media are supplemented with tryptophan (32, 35). *Pseudomonas putida* and *Pseudomonas fluorescens* isolated from plant rhizospheres produce a similar profile of indole compounds from tryptophan as we found for pseudomonads in our study, with *P. putida* producing IAA, ILA, IAM, and IAld and *P. fluorescens* producing IAA, ILA, and IAld (25).

Two pathways for IAA synthesis from L-tryptophan may be functional in *X. campestris* pv. *glycines* and *P. syringae* pv. *phaseolicola* and pv. *syringae*. The first, which is common to many microbes and plants, involves transamination, converting tryptophan to indole-3-pyruvic acid (IPyA), followed by decarboxylation of IPyA to indole-3-acetaldehyde (IAAld) and finally oxidation of IAAld to IAA (30, 33). Even though we failed to detect the intermediates IPyA and IAAld, we did detect ILA, which is a common product of reduction of IPyA (33). The other pathway, common both to

A. tumefaciens, which causes neoplastic growth on numerous dicots, and *P. syringae* pv. *savastanoi*, which causes hyperplastic growth on olive, oleander, privet, and ash, involves decarboxylation by a monooxygenase to IAM followed by deamination by a hydrolase to give IAA (17, 29, 38, 40). The genes for IAA production through this pathway are carried on the Ti plasmid of *A. tumefaciens* and are expressed in the host plant after transfer of the T-DNA segment to the plant genome (29, 38, 40). For *P. syringae* pv. *savastanoi* the corresponding genes can be located either on a plasmid or on the chromosome (4). These two bacteria are also capable of synthesizing IAA from tryptophan via IPyA and IAAld (15, 19). *P. putida* and *P. fluorescens* may also be able to utilize both pathways for IAA synthesis (25).

When disease symptoms include hypertrophy of host tissue, the involvement of increased auxin levels in the disease process is a priori very likely. However, IAA has numerous other effects on plant tissue which may favor bacterial multiplication in planta in the absence of hypertrophy. One such effect, which has been proposed to be important for the growth of tumors incited by *A. tumefaciens*, is the promotion of solute uptake due to modification of the electrochemical proton gradient across the host plasmalemma (26). Other effects which may affect the outcome of plant-bacteria interactions include an increase in respiration, protein synthesis, activities of various enzymes including cellulase, protein secretion, and release of cell wall polymer and the induction of ethylene synthesis (30, 45).

Much further research is required to determine whether the pseudomonads and xanthomonads studied here produce IAA in their hosts and, if so, to determine whether in planta IAA production is a virulence determinant. The possibility also exists that IAA production by phytopathogenic bacteria contributes to their ability to survive as epiphytes on plant surfaces.

LITERATURE CITED

1. **Badenoch-Jones, J., R. E. Summons, B. Entsch, B. G. Rolfe, C. W. Parker, and D. S. Letham.** 1982. Mass spectrometric identification of indole compounds produced by *Rhizobium* strains. Biomed. Mass Spectrom. **9**:429–437.
2. **Barea, J. M., and M. E. Brown.** 1974. Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. J. Appl. Bacteriol. **37**:583–593.
3. **Brugger, B. B., and N. T. Keen.** 1979. Specific elicitors of glyceollin accumulation in the *Pseudomonas glycinea* soybean host-parasite system. Physiol. Plant Pathol. **15**:43–51.
4. **Comai, L., G. Surico, and T. Kosuge.** 1982. Relation of plasmid DNA to indoleacetic acid production in different strains of *Pseudomonas syringae* pv. *savastanoi*. J. Gen. Microbiol. **128**:2157–2163.
5. **Ek, M., P. O. Ljungquist, and E. Stenström.** 1983. Indole-3-acetic acid production by mycorrhizal fungi determined by gas chromatography-mass spectrometry. New Phytol. **94**:401–407.
6. **Erikson, D.** 1944. Certain aspects of resistance of plum trees to bacterial canker. Part I. Some biochemical characteristics of *Pseudomonas morsprunorum* (Wormald) and related phytopathogenic bacteria. Ann. Appl. Biol. **32**:44–52.
7. **Fett, W. F.** 1984. Accumulation of isoflavonoids and isoflavone glucosides after inoculation of soybean leaves with *Xanthomonas campestris* pv. *glycines* and pv. *campestris* and a study of their role in resistance. Physiol. Plant Pathol. **24**:303–320.
8. **Galachyan, R. M.** 1969. Nature of metabolites of microorganisms producing plant tumors as root-promoting substances. Voprosy Mikrobiologii **4**:129–142. (In Russian with English summary.)
9. **Gordon, S. A., and R. P. Weber.** 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. **26**:192–195.
10. **Groth, D. E., and E. J. Braun.** 1986. Growth kinetics and histopathology of *Xanthomonas campestris* pv. *glycines* in leaves of resistant and susceptible soybeans. Phytopathology **76**:959–965.
11. **Gruen, H. E.** 1959. Auxins and fungi. Annu. Rev. Plant Physiol. **10**:405–440.

11a. **Haas, M. J., and W. F. Fett.** 1987. Detection of plasmids in the plant pathogenic bacterium *Xanthomonas campestris* pathovar *glycines*, p. 148–154. *In* M. J. Comstock (ed.), Biotechnology in agricultural chemistry. American Chemical Society, Washington, D.C.

12. **Hirata, S.** 1960. Studies on the phytohormone in the malformed portion of the diseased plants. 10. Indole-acetic acid production of five species of bacteria causing gall, canker or scab. Miyazaki Deigaku Nogaku Kenkyu Jiho **5**:85–92. (In Japanese with English summary.)
13. **Jamieson, W. D., and O. Hutzinger.** 1970. Identification of simple naturally occurring indoles by mass spectrometry. Phytochemistry **9**:2029–2036.
14. **Jones, S. B., and W. F. Fett.** 1987. Bacterial pustule disease of soybean: microscopy of pustule development in a susceptible cultivar. Phytopathology **77**:266–274.
15. **Kaper, J. M., and H. Veldstra.** 1958. On the metabolism of tryptophan by *Agrobacterium tumefaciens*. Biochim. Biophys. Acta **30**:401–420.
16. **Koizumi, M.** 1977. Behaviour of *Xanthomonas citri* (Hasse) Dowson and histological changes in diseased tissues in the process of lesion extension. Ann. Phytopathol. Soc. Jpn. **43**: 129–136.
17. **Kosuge, T., M. G. Heskett, and E. E. Wilson.** 1966. Microbial synthesis and degradation of indole-3-acetic acid. I. The conversion of L-tryptophan to indole-3-acetamide by an enzyme system from *Pseudomonas savastanoi*. J. Biol. Chem. **241**: 3738–3744.
18. **Krebs, K. G., D. Heusser, and H. Wimmer.** 1969. Spray reagents, p. 854–909. *In* E. Stahl (ed.), Thin-layer chromatography. Springer-Verlag, New York.
19. **Kuo, T. T., and T. Kosuge.** 1970. Role of aminotransferase and indole-3-pyruvic acid in the synthesis of indole-3-acetic acid in *Pseudomonas savastanoi*. J. Gen. Appl. Microbiol. **16**:191–204.
20. **Liao, C. H., and J. M. Wells.** 1987. Association of pectolytic strains of *Xanthomonas campestris* with soft-rots of fruits and vegetables at retail markets. Phytopathology **77**:418–422.

20a. **Liao, C. H., and J. M. Wells.** 1987. Diversity of Pectolytic, fluorescent pseudomonads causing soft rots at retail markets. Phytopathology **77**:673–677.

21. **Liu, S.-T., and C. I. Kado.** 1979. Indoleacetic acid production: a plasmid function of *Agrobacterium tumefaciens* C58. Biochem. Biophys. Res. Commun. **90**:171–178.
22. **Liu, S. T., K. L. Perry, C. L. Schardl, and C. I. Kado.** 1982. *Agrobacterium* Ti plasmid indoleacetic acid gene is required for crown gall oncogenesis. Proc. Natl. Acad. Sci. USA **79**:2812–2816.
23. **Loper, J. E., and M. N. Schroth.** 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology **76**:386–389.
24. **Offringa, I. A., L. S. Melchers, A. J. G. Regenburg-Tuink, P. Costantino, R. A. Schilperoort, and P. J. J. Hooykaas.** 1986. Complementation of *Agrobacterium tumefaciens* tumor-inducing *aux* mutant by genes from the T_R-region of the Ri plasmid of *Agrobacterium rhizogenes*. Proc. Natl. Acad. Sci. USA **83**: 6935–6939.
25. **Prikryl, Z., V. Vancura, and M. Wurst.** 1985. Auxin formation by rhizosphere bacteria as a factor in root growth. Biol. Plant. **27**:159–163.
26. **Rausch, T., G. Kahl, and W. Hilgenberg.** 1984. Primary action of indole-3-acetic acid in crown gall tumors. Increase in solute uptake. Plant Physiol. **75**:354–358.
27. **Rigaud, J.** 1970. L'acide indolyl-3-lactique et son metabolisme chez *Rhizobium*. Arch. Mikrobiol. **72**:297–307.
28. **Robeson, D. J., and D. R. Cook.** 1985. Biotransformation of tryptophan to indole-3-acetic acid by *Xanthomonas campestris* pv. *campestris*. Z. Naturforsch. Teil C **40**:740–742.

29. **Schröder, G., S. Waffenschmidt, E. W. Weiler, and J. Schröder.** 1984. The T-region of Ti plasmids codes for an enzyme synthesizing indole-3-acetic acid. Eur. J. Biochem. **138**:387–391.
30. **Sembdner, G., D. Gross, H. W. Liebisch, and G. Schneider.** 1980. Biosynthesis and metabolism of plant hormones, p. 281–444. *In* J. MacMillan (ed.), Hormonal regulation of development. I. Molecular aspects of plant hormones. Springer-Verlag, New York.
31. **Sequeira, L.** 1973. Hormone metabolism in diseased plants. Annu. Rev. Plant Physiol. **24**:353–380.
32. **Sequeira, L., and P. H. Williams.** 1964. Synthesis of indoleacetic acid by *Pseudomonas solanacearum*. Phytopathology **54**:1240–1246.
33. **Sheldrake, A. R.** 1973. The production of hormones in higher plants. Biol. Rev. Camb. Philos. Soc. **48**:509–559.
34. **Sinclair, J. B., and M. C. Shurtleff.** 1975. Compendium of soybean diseases. The American Phytopathological Society, St. Paul, Minn.
35. **Smidt, M., and T. Kosuge.** 1978. The role of indole-3-acetic acid accumulation by alpha methyl tryptophan-resistant mutants of *Pseudomonas savastanoi* in gall formation on oleanders. Physiol. Plant Pathol. **13**:203–214.
36. **Souw, P., and A. L. Demain.** 1979. Nutritional studies on xanthan production by *Xanthomonas campestris* NRRL B1459. Appl. Environ. Microbiol. **37**:1186–1192.
37. **Surico, G., N. S. Iacobellis, and A. Sisto.** 1985. Studies on the role of indole-3-acetic acid and cytokinins in the formation of knots on olive and oleander plants by *Pseudomonas syringae* pv. *savastanoi*. Physiol. Plant Pathol. **26**:309–320.
38. **Thomashow, M. F., S. Hugly, W. G. Buchholz, and L. S. Thomashow.** 1986. Molecular basis for the auxin-independent phenotype of crown gall tumor tissues. Science **231**:616–618.
39. **Tien, T. M., M. H. Gaskins, and D. H. Hubbell.** 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. **37**:1016–1024.
40. **Van Onckelen, H., E. Prinsen, D. Inźe, P. Rüdelsheim, M. Van Lijsebettens, A. Follin, J. Schell, M. Van Montagu, and J. DeGreef.** 1986. *Agrobacterium* T-DNA gene 1 codes for tryptophan 2-monooxygenase activity in tobacco crown gall cells. FEBS Lett. **198**:357–360.
41. **Wichner, S., and E. Libbert.** 1968. Interactions between plants and epiphytic bacteria regarding their auxin metabolism. I. Detection of IAA-producing epiphytic bacteria and their role in long duration experiments on tryptophan metabolism in plant homogenates. Physiol. Plant. **21**:227–241.
42. **Wimalajeewa, D. L. S., and I. G. Pascoe.** 1978. Bacterial stem canker of loquat. Aust. Plant Pathol. **7**:33.
43. **Wolf, F. A.** 1924. Bacterial pustule of soybean. J. Agri. Res. **29**:57–68.
44. **Yokata, T., N. Murofushi, and N. Takahashi.** 1980. Extraction, purification, and identification, p. 113–201. *In* J. MacMillan (ed.), Hormonal regulation of development. I. Molecular aspects of plant hormones. Springer-Verlag, New York.
45. **Zeroni, M., and M. A. Hall.** 1980. Molecular effects of hormone treatment on tissue, p. 511–586. *In* J. MacMillan (ed.), Hormonal regulation of development. I. Molecular aspects of plant hormones. Springer-Verlag, New York.